Supplementary information

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Includes: Figures S1-S4

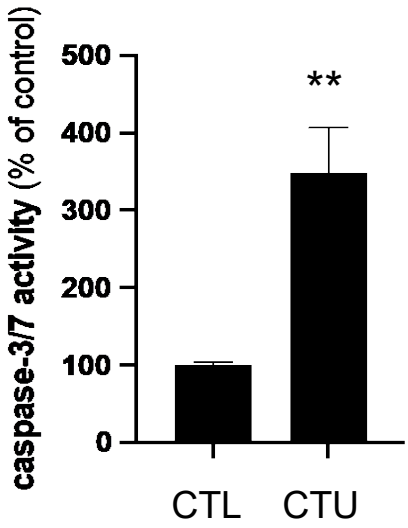


Figure S1: Increased caspase-3/7 activity produced in MDA-MB-231 cells by CTU. MDA-MB-231 cells were seeded in duplicate (7 x 10^3 cells/well) in black-walled 96-well plates and incubated at 37°C for 24 h. The medium was removed and cells were incubated for a further 24 h in serum-free medium before treatment with CTU (10 μM) for 24 h. Luminescence was measured using Caspase-Glo 3/7 assay according to the manufacturer's instructions (Promega, Madison, WI) in a SpectraMax iD5 microplate reader (Molecular Devices, San Jose, CA). Experiments were conducted on three separate occasions. The data are expressed as means ± standard deviations. Different from control (CTL): **P<0.01 (unpaired Student's t-test).

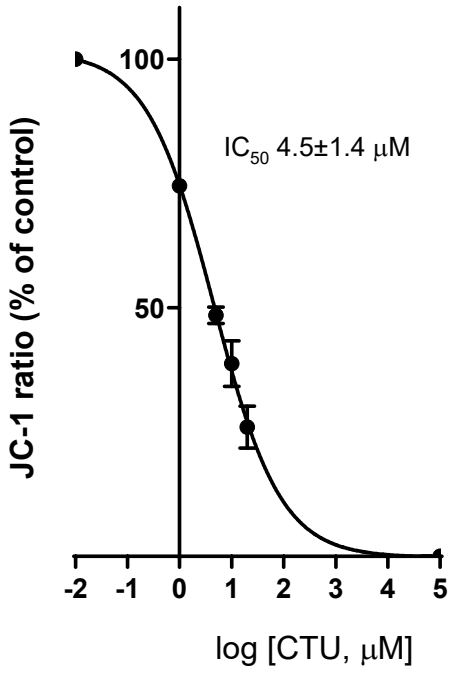


Figure S2: MDA-MB-231 cells were seeded in triplicate on 96-well microplates (7 x 10^3 cells/well). Twenty-four h after serum removal, cells were treated with different concentrations of CTU for 4 h, then incubated with JC-1 (1.5 μM) in serum-free medium for 20 min. Following serum removal, cells were washed with PBS and the fluorescence of JC-1 aggregates and monomers was measured at

excitation/emission wavelengths of 535/595 nm and 485/535 nm, respectively, in a Fluoroskan Ascent FL micro-plate reader (Labsystems, Upplands Väsby, Stockholm County, Sweden). The IC_{50} was determined by non-linear regression of the plot of $\log_{10}$ [CTU] versus % of control JC-1 ratio (GraphPad Prism 8).

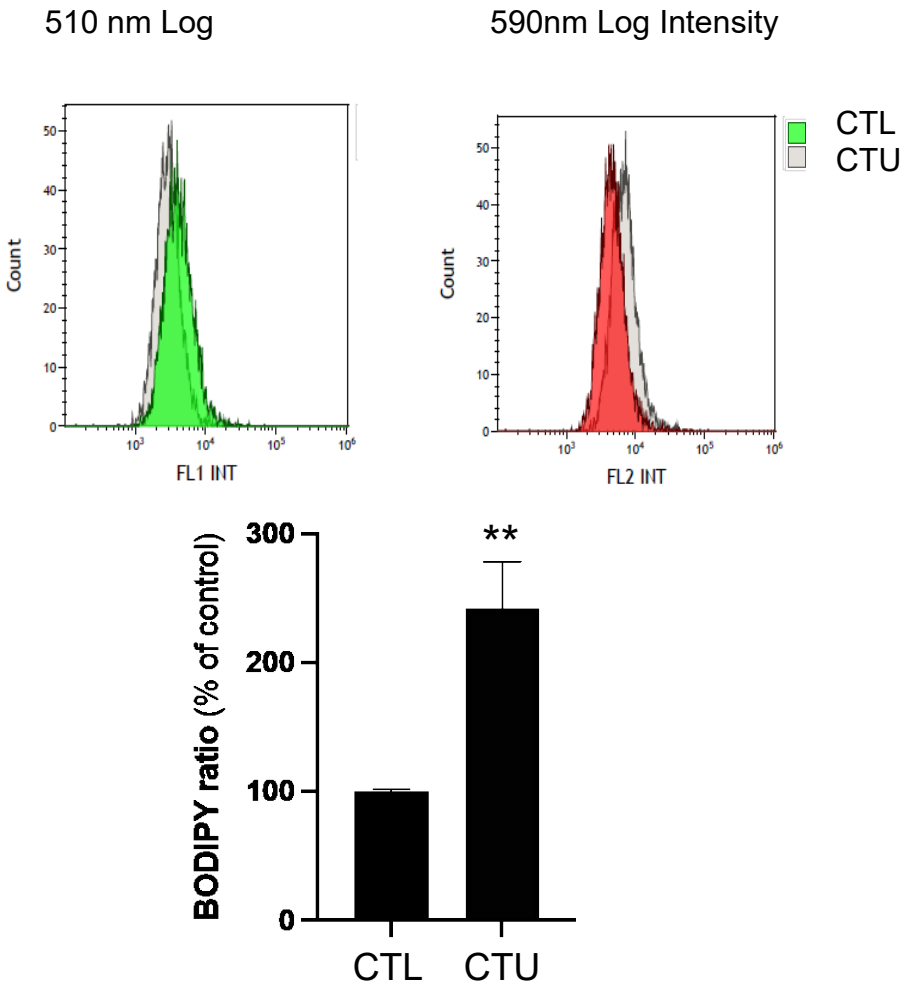


Figure S3: ROS production in MDA-MB-231 cells after treatment with CTU (10 µM, 24 h) was detected using C11-BODIPY (581/591). MDA-MB-231 cells (7.5 x 10^4/well) were seeded in duplicate on 6-well plates and, 24 h after serum removal, were treated with CTU (10 µM, 4 h). Cells were then incubated with BODIPY (581/591) dye (1 µM, 30 min). Cells were trypsinized, washed and resuspended in PBS (500 µL) for analysis in a Gallios flow cytometer using Kaluza software (Beckman Coulter, Lane Cove West, NSW, Australia). Experiments were conducted on three separate occasions. The data are expressed as means ± SD. Different from CTL: **P<0.01 (unpaired Student's t-test).

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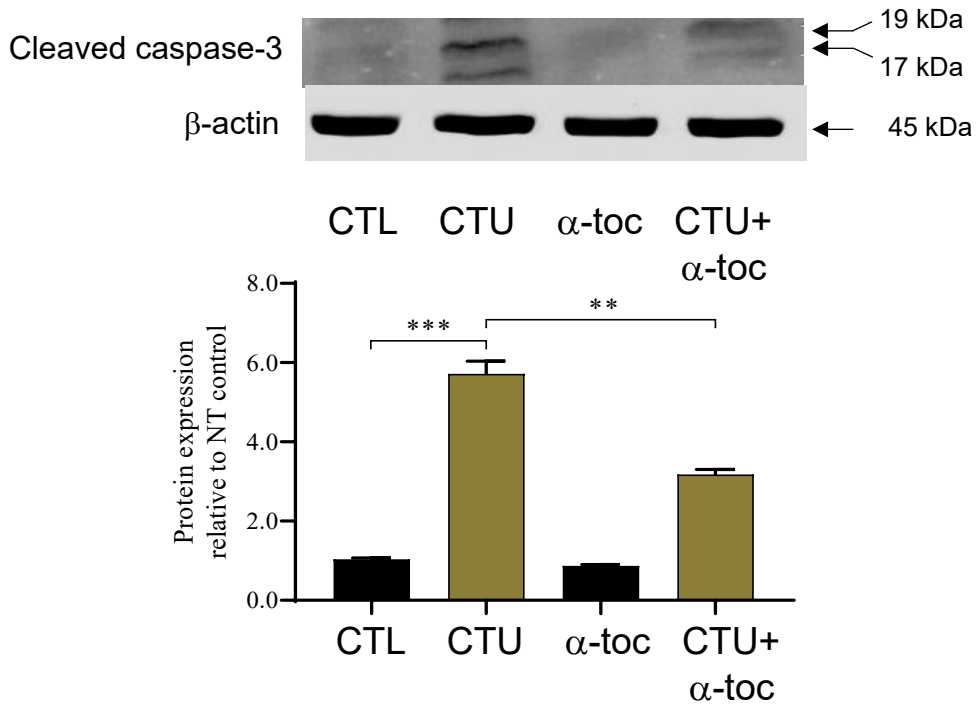


Figure S4: CTU-mediated increases in immunoreactive cleaved caspase-3 were normalized by pretreatment of MDA-MB-231 cells with α-tocopherol (α-toc, 100 μM, 4 h). Experiments were conducted on three separate occasions. The data are expressed as means ± SD. Data were analysed using one-way ANOVA in combination with Tukey's post hoc test. Different from CTU alone: **P<0.01, ***P<0.001.